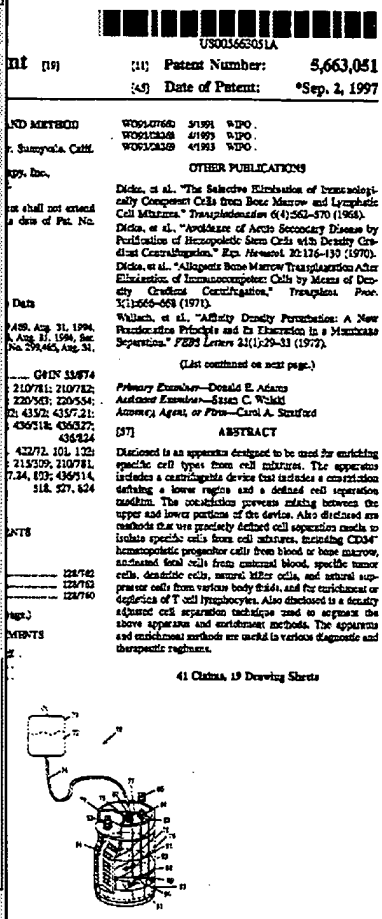
ml/mln for 200 min (total volume of 16 L). Peripheral blood buffy coats from healthy donors prepared by conventional methods were obtained from Stanford University Hospital Blood Bank, Palo Alto, Calif.

Detailed Description Text - DETX (284):

In instances where the dendritic cells are used to generate peptide-specific cytotoxic T lymphocytes (CTL) for purposes of elucidating their antigen presentation function, the interface fraction (mostly monocytes) is resuspended in cold pooled human AB serum (Irvine Scientific, Santa Ana, Calif.) to which an equal volume of 80% AB serum 20% dimethyl sulfoxide (DMSO) (Sigma Chemical Company, St. Louis, Mo.) is added dropwise. The resulting cell suspension is aliquoted into cryovials and frozen in liquid nitrogen. The monocytes can be used for restimulation of CTL for expansion.



US005663051A

[19] [11] Patent Number: 5,663,051

[45] Date of Patent: *Sep. 2, 1997

WO9107660 5/1991 WIPO .
WO9123368 4/1992 WIPO .
WO9123369 4/1992 WIPO .

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Dicke, et al., "The Selective Elimination of Immunologically Competent Cells from Bone Marrow and Lymphatic Cell Mixtures," *Transplantation* 6(4):562-570 (1968).

Dicke, et al., "Apoldasse of Acute Secondary Disease by Purification of Hemopoietic Stem Cells with Density Gradient Centrifugation," *Exp. Hematol.* 32:126-130 (1970).

Dicke, et al., "Allogenic Bone Marrow Transplantation After Elimination of Immunocompetent Cells by Means of Density Gradient Centrifugation," *Transplant. Proc.* 3(1):665-668 (1971).

Wallach, et al., "Affinity Density Fractionation: A New Fractionation Principle and Its Elaboration in a Membrane Separation," *FEBS Letters* 21(1):29-33 (1972).

(List continued on next page.)

Primary Examiner—Donald E. Adams
Assistant Examiner—Susan C. Wicklil
Attorney, Agent, or Firm—Carol A. Stanford

[57] ABSTRACT

Disclosed is an apparatus designed to be used for enriching specific cell types from cell mixtures. The apparatus includes a centrifugable device that includes a constriction defining a lower region and a defined cell separation medium. The constriction prevents mixing between the upper and lower portions of the device. Also disclosed are methods that use precisely defined cell separation media to isolate specific cells from cell mixtures, including CD34+ hemopoietic progenitor cells from blood or bone marrow, activated fetal cells from maternal blood, specific tumor cells, dendritic cells, natural killer cells, and natural suppressor cells from various body fluids, and for enrichment or depletion of T cell lymphocytes. Also disclosed is a density adjusted cell separation technique used to segment the above apparatus and enrichment methods. The apparatus and enrichment methods are useful in various diagnostic and therapeutic regimens.

41 Claims, 19 Drawing Sheets

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completely, depleted of CD19+ cells or, alternatively, CD34+ cells could be obtained from cord or peripheral blood, where the population of CD19+ cells is greatly reduced.

Detailed Description Text - DETX (23):
The neutrophil precursor cells possibly may be frozen in liquid nitrogen for long periods of storage. The cells then may be thawed and used as needed.

Detailed Description Text - DETX (24):
Cryoprotective agents, which can be used, include but are not limited to dimethyl sulfoxide (DMSO) (Lovelock, J. E. and Bishop, M. W. H., 1959, Nature 183:1394-1395; Ashwood-Smith, M. J., 1961, Nature 190:1204-1205), hetastarch glycerol, polvinylpyrrolidine (Rinfret, A. P., 1960, Ann. N.Y. Acad. Sci. 85:576), polyethylene glycol (Sloviter, H. A. and Ravdin, R. G., 1962, Nature 196:548), albumin, dextran, sucrose, ethylene glycol, i-erythritol, D-ribitol, D-mannitol (Rowe, A. W., et al., 1962, Fed. Proc. 21:157), D-sorbitol, i-inositol, D-lactose, choline chloride (Bender, M. A., et al., 1960, J. Appl. Physiol. 15:520), amino acids (Phan The Tran and Bender, M. A., 1960, Exp. Cell Res. 20:651), methanol, acetamide, glycerol monoacetate (Lovelock, J. E., 1954, Biochem. J. 56:265), and inorganic salts (Phan The Tran and Bender, M. A., 1960, Proc. Soc. Exp. Biol. Med. 104:388; Phan The Tran and Bender, M. A., 1961, in Radiobiology, Proceedings of the Third Australian Conference on Radiobiology, Ilbery, P. L. T., ed., Butterworth, London, p. 59).

Detailed Description Text - DETX (25):
Typically, the cells may be stored in 10% DMSO, 50% serum, and 40% RPMI 1640 medium. Once thawed, the cells may be induced to proliferate and further differentiate by the introduction of the appropriate hematopoietic growth factors.

Detailed Description Paragraph Table - DETL (2):
TABLE II ________________________________ PROLIFERATION AND CFC PRESENT IN CD11b/CD15 PHENOTYPES FROM UMBILICAL CORD BLOOD ENRICHED CD34+ CELLS Experiment Number 1 2 3 4 ________________________________ % CD34 45 80 80 59 Day of Culture 11 14 9 9 Fold Change Cell # *72 54 72 34 Region A (11b- **5.7 2 ND ND 15-) fold change CFU-GM ***19 14 8 29 CFU-M 38 14 25 97 BFU-E 17 14 39 60 CFU-MIX 0 0 6 22 Cloning Efficiency 0.55 0.42 0.78 2.08 Region B (11b- 9.5 12 ND ND 15+) fold change CFU-GM 41 64 11 27 CFU-M 69 188 295 246 BFU-E 0 0 0 0 CFU-MIX 0 0 0 0 Cloning Efficiency 1.1 2.52 3.06 2.73 Region C (11b+ 1.1 ND ND ND 15+) fold change CFU-GM 0 ND 9 0 CFU-M 0 ND 0 0 BFU-E 0 ND 0 0 CFU-MIX 0 ND 0 0 Cloning Efficiency 0 0 0.09 0 ________________________________ ND = not determined *Fold increase in cell number during initial culture period **Fold increase in cell number from the sorted phenotype after an additio 7 days of culture ***Colonies per 10 4 cells of the sorted phenotype

US005700691A

[11] Patent Number: 5,700,691

[45] Date of Patent: Dec. 23, 1997

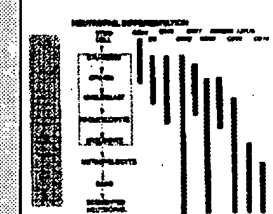
(List continued on next page.)

Primary Examiner—Brian R. Stanton
Attorney, Agent, or Firm—Campbell & Flores LLP

[57] ABSTRACT

A composition of human neutrophil precursor cells is disclosed wherein at least 16% of the cells are human myeloblasts and promyelocytes. The myeloblasts and promyelocytes are derived from human neutrophil progenitor cells that were obtained from peripheral blood, bone marrow or cord blood. The neutrophil precursor cells consists less than 5% colony forming units. Also disclosed are human neutrophil precursor cells made up of about 16% CD15+CD11b- cells and less than 5% colony forming units and methods of preparing these compositions.

1 Claim, 3 Drawing Sheets



anti-coagulant-containing tubes or by apheresis or leukopheresis. Complete blood does not need to be processed or diluted prior to centrifugation. However, since the methods enrich fetal cells based on their specific buoyant density, it is important that the cells are subject to separation within a relatively short time after collection, because the density of the cells changes according to their culture or storage conditions. Therefore, in order to obtain optimal enrichment of fetal cells from maternal blood, it is preferred that the blood samples are used within 48 hours after their collection. Most preferably, blood samples should be subjected to density gradient centrifugation within several hours of collection.

Detailed Description Text - DETX (122):

For enrichment of CD34.sup.+ cells from blood, such as peripheral or umbilical cord blood, the cell separation medium should be adjusted to a density of 1.0605.+-.0.0005 gr/ml, a physiologic osmolality of 270-290 mOsm/kg H.sub.2 O and physiologic pH 6.8-7.8. More preferably, the density will be 1.0605.+-.0.0002 gr/ml, and the osmolality will be 280 mOsm/kg H.sub.2 O, at pH 7.4.

Detailed Description Text - DETX (155):

A major advantage of the methods described herein is that a large volume of complete blood may be directly placed on the density gradient. Peripheral blood may be collected in anti-coagulant-containing tubes or by apheresis or leukopheresis. Complete blood does not need to be processed or diluted prior to centrifugation. However, since the methods enrich breast tumor cells based on their specific buoyant density, it is important that the cells are subject to separation within a relatively short time after their collection from an in vivo source because the density of the cells changes according to their culture or storage conditions. Therefore, in order to obtain optimal enrichment of breast tumor cells from blood, it is preferred that the blood samples are used within 48 hours after their collection. Most preferably, blood samples should be subjected to density gradient centrifugation within several hours of collection.

Detailed Description Text - DETX (207):

Patients were hydrated and treated with cyclophosphamide (4 gm/m.sup.2) administered by intravenous (IV) infusion over two hours through a central venous catheter. Twenty-four hours after the completion of the cyclophosphamide infusion, patients were treated with G-CSF (Neupogen, Amgen, Thousand Oaks, Calif.) administered by subcutaneous (SC) injection at a dose of approximately 10 .mu.g/kg/d. Apheresis was initiated upon recovery of the white blood cell count (WBC) to equal or more than 1.times.10.sup.9 /L. Apheresis was performed using a Cobe Spectra Cell Separator (Lakewood, Colo.) at a rate of 80 ml/mln for 200 min (total volume of 16 L). Peripheral blood buffy coats from healthy donors prepared by conventional methods were obtained from Stanford University Hospital Blood Bank, Palo Alto, Calif.

US005663051A

[11] Patent Number: 5,663,051

[45] Date of Patent: *Sep. 2, 1997

OTHER PUBLICATIONS

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Dicke, et al., "Prevention of Acute Secondary Disease by Purification of Hemopoietic Stem Cells with Density Gradient Centrifugation." *Exp. Hematol.* 20:126-130 (1970).

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Willach, et al., "Affinity Density Perturbation: A New Fractionation Principle and Its Elucidation in a Membrane Separation." *FEBS Letters* 21(1):29-33 (1972).

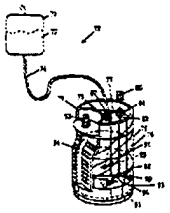
(List continued on next page.)

Primary Examiner—Donald E. Adams
Assistant Examiner—Susan C. Wicklll
Attorney, Agent, or Firm—Carol A. Stanford

[57] ABSTRACT

Disclosed is an apparatus designed to be used for enriching specific cell types from cell mixtures. The apparatus includes a centrifugable device that includes a centrifuge defining a lower region and a defined cell separation medium. The centrifuge prevents mixing between the upper and lower portions of the device. Also disclosed are methods that use precisely defined cell separation media to isolate specific cells from cell mixtures, including CD34+ hematopoietic progenitor cells from blood or bone marrow, enclosed fetal cells from maternal blood, specific tumor cells, dendritic cells, natural killer cells, and natural suppressor cells from various body fluids, and for enrichment or depletion of T cell lymphocytes. Also disclosed is a density adjusted cell separation technique used to segment the above apparatus and enrichment methods. The apparatus and enrichment methods are useful in various diagnostic and therapeutic regimens.

41 Claims, 19 Drawing Sheets



US-PAT-NO: 5486359

DOCUMENT-IDENTIFIER: US 5486359 A

TITLE: Human mesenchymal stem cells

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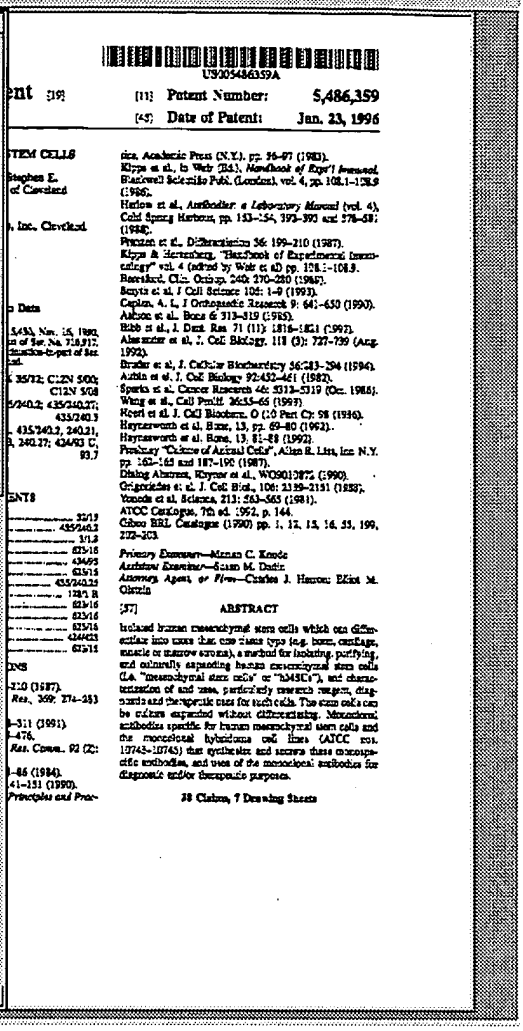
Brief Summary Text - BSTX (8):
In order to obtain subject human mesenchymal stem cells, it is necessary to isolate rare pluripotent mesenchymal stem cells from other cells in the bone marrow or other MSC source. Bone marrow cells may be obtained from iliac crest, femora, tibiae, spine, rib or other medullary spaces. Other sources of human mesenchymal stem cells include embryonic yolk sac, placenta, umbilical cord, fetal and adolescent skin, and blood.

Detailed Description Text - DETX (60):
Marrow cells from either the femoral head cancellous bone or the iliac aspirate were cultured in complete medium (i.e. BGI.sub.b medium with 10% fetal bovine serum) at 37.degree. C. in humidified atmosphere containing 95% air and 5% CO.sub.2. In preliminary experiments the cells were allowed to attach for 1, 3, or 7 days prior to the initial medium change. No increase in cell attachment was observed after day 1, therefore, one day was chosen as the standard length of time at which nonadherent cells were removed from the cultures by replacing the original medium with 7 ml of fresh complete medium. Subsequent medium changes were performed every 4 days. When culture dishes became confluent, the cells were detached with 0.25% trypsin with 0.1 mM EDTA (GIBCO) for 10-15 minutes at 37.degree. C. The action of trypsin was stopped with 1/2 volume fetal bovine serum. The cells were counted, split 1:3, and replated in 7 ml complete medium. Aliquots of cells were cryopreserved in 90% fetal bovine serum with 10% DMSO (freezing medium).

Detailed Description Text - DETX (135):
Cell Freezing Medium at 37.degree. C.

Detailed Description Text - DETX (236):
12. Sodium azide was then added so that its final concentration was 0.02%. This ascitic fluid was then stored in small aliquots at -70.degree. C. The stability of each antibody to freezing and thawing was tested before the entire ascites prep was frozen.

Detailed Description Text - DETX (250):



US005486359A

[11] Patent Number: 5,486,359

[45] Date of Patent: Jan. 23, 1996

[57] ABSTRACT

Isolated human mesenchymal stem cells which can differentiate into more than one tissue type (e.g. bone, cartilage, muscle or marrow stroma), a method for isolating, purifying, and culturally expanding human mesenchymal stem cells (i.e. "mesenchymal stem cells" or "hMSC"), and characterization of and uses, particularly research reagent, diagnostic and therapeutic uses for such cells. The stem cells can be culture expanded without differentiating. Monoclonal antibodies specific for human mesenchymal stem cells and the monoclonal hybridoma cell lines (ATCC nos. 10745-10745) that synthesize and secrete these monospecific antibodies, and uses of the monoclonal antibodies for diagnostic and/or therapeutic purposes.

38 Claims, 7 Drawing Sheets